Applicant: Robert W. Busby et al.

Serial No.: 09/800,863 Filed

: March 7, 2001

Page

: 5

Attorney's Docket No.: 14184-008002

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 76, line 18 has been amended as follows:

RHO1 coding sequence for construction of dominant mutations can be isolated from Saccharomyces cerevisiae genomic DNA. Primers 5'cgcGGATCCCGACATATTCGAGGTTGACT-3' (SEQ ID NO: 11) and 5'cccAAGCTTGCTAGAAATATGAACCTTCC-3' (SEQ ID NO: 12) are used to amplify RHO1 coding sequence with 1 kilobase of upstream regulatory sequence and 500 basepairs of downstream regulatory sequence. BamHI and HindIII restriction sites are added to the oligonucleotides to facilitate cloning into the pRS416 centromere-based yeast vector. The Quik Change Site-Directed Mutagenesis Kit (Stratagene, La Jolla CA) is used to first create a mutation that encodes the G22S substitution; next, the pRS416rho1G22S plasmid is used as a template to introduce a mutation that encodes the D125N substitution. Primer pair 5'-gtgcctgtAgtaagacatgt-3' (SEQ ID NO: 441)/ 5'-acatgtcttacTacaggcac-3'(SEQ ID NO: 442) is used to anneal to the pRS416RHO1 template for pRS416rho1G22S allele construction. Primer pair 5'gtaaagtgAatttgagaaac-3' (SEQ ID NO: 443)/ 5'- gtttctcaaatTcactttac-3' (SEO ID NO: 444) is used to anneal to the pRS416rho1G22S template for pRS416rho1G22S D125N allele construction. pRS416rho1G22S D125N and control plasmids (pRS416RHO1 and pRS416) are then used to transform a wild-type ura3 auxotrophic strain. Transformants are selected and grown at 25°C in synthetic liquid growth medium lacking uracil and containing the osmolyte sorbitol (1M). Cultures are then transferred to growth in synthetic liquid growth medium lacking uracil without sorbitol, and cells are visually inspected following growth for various periods of time. Expression of the rho1G22S D125N dominant allele causes cell lysis after growth for approximately 120 minutes.

Paragraph beginning at page 78, line 28 has been amended as follows:

Applicant: Robert W. Busby et al

Serial No.: 09/800,863 Filed

: March 7, 2001

Page

: 6

Attorney's Docket No.: 14184-008002

RSR1 coding sequence for construction of dominant mutations can be isolated from Saccharomyces cerevisiae genomic DNA. Primers 5'-

cgcGGATCCTATCTTCACTCAATATACTTCCTA-3' (SEO ID NO: 17) and 5'cccAAGCTTCATCGTTGAAACTTGATAACGCAC-3' (SEQ ID NO: 18) are used to amplify RHO1 coding sequence with 750 basepairs of upstream regulatory sequence and 500 basepairs of downstream regulatory sequence. BamHI and HindIII restriction sites are added to the oligonucleotides to facilitate cloning into the pRS416 centromere-based yeast vector. The Quik Change Site-Directed Mutagenesis Kit (Stratagene, La Jolla CA) is used to create dominantnegative RSR1 substitution mutation K16N. Primer pair 5'- tggtgtcggtaaTtcctgcttaac-3' (SEQ ID NO: 445) / 5'- gttaagcaggaAttaccgacacca-3' (SEQ ID NO: 446) is used to anneal to the pRS416RSR1 template for allele construction. The pRS416rsr1K16N and control pRS416 plasmids are then used to transform a haploid wild-type *ura3* auxotrophic strain. Transformants are selected and grown at 30°C in YPD liquid growth medium. Log phase cultures are fixed in 3.7% formaldehyde (vol:vol) and stained with the chitin-binding dye Calcofluor white, as described; previous sites of bud formation are marked with a chitin-rich structure called a bud scar. Fluorescent microscopy reveals that cells containing the control plasmid display clustering of bud scars at one pole of the cells, the well-characterized haploid pattern of bud site selection. Cells expressing rsr1K16N display a random pattern of bud site selection; bud scars are scattered across the surface of haploid cells. Cells expressing rsr1K16N do not display other obvious growth or morphological defects.

Table 1 page 1 has been amended as follows:

SEO ID NOs: 21-440 respectively Gene **Organisms**